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Cardiff Road
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1. Your reference
474MCE98

2. Patent application number
(The Patent Office will fill in this part)
17 SEP 1998 **9820163.5**

3. Full name, address and postcode of the or of each applicant *(underline all surnames)*
SENTEC LTD
TERRINGTON HOUSE
13-15 HILLS ROAD
CAMBRIDGE
CB2 1GE

Patents ADP number *(if you know it)* 7353733002

If the applicant is a corporate body, give the country/state of its incorporation

4. Title of the invention
MICRO-FABRICATED CODED LABELS, READING SYSTEMS AND THEIR APPLICATIONS

5. Name of your agent *(if you have one)*
MR A. HOWE
SENTEC LTD
TERRINGTON HOUSE
13-15 HILLS ROAD
CAMBRIDGE CB2 1GE

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

Patents ADP number *(if you know it)* 7464621001

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and *(if you know it)* the or each application number

Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*
NO		

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Statement of inventorship and right to grant of a patent *(Patents Form 7/77)*	1 ✓
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Any other documents *(please specify)*	NONE

11. I/We request the grant of a patent on the basis of this application.

Signature [signature] Date 15/9/98

12. Name and daytime telephone number of person to contact in the United Kingdom 01223 303800 MR ANDREW HOWE

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Micro-fabricated coded labels, reading systems and their applications

This invention relates to micro-fabricated tags, methods for making them, reading them and using them.

Background and existing art

Massively parallel bioassay tests are the enabling techniques that have made the majority of recent advances in genetics, screening and drug discovery possible. Thousands or millions of tests that previously were carried out one by one are now combined into a single experiment yielding thousands or millions of results. The key to this process has been the development of techniques that allow the results of the many different tests to be separated from each other.

Currently there are a limited number of these techniques, all are costly to use, and none is universally applicable. The techniques consist of labelling each of the constituent experiments in a manner that can be read after the experiment has completed. Labels used at present include the position of the experiment on the surface of a test chip and the fluorescent spectrum of a particle to which the experiment is bound.

The present market is dominated by US companies. Affymetrix markets a system called the GeneChip probe array. This is a DNA sequence testing chip, where tens of thousands of different DNA probes are located at known points on a large 2D array. Standard DNA hybridisation techniques are used in a chamber above the chip, and the test results are read out optically by the positions of fluorescent dots on the array. Some disadvantages are:

- High cost. A single GeneChip is very expensive, because the fabrication process is non-standard and highly complex, and the chips are in great demand. The readers are expensive with complex imaging optics.
- Single use. Once used, Affymetrix recommend that the GeneChip (12.5×12.5 mm^2) is thrown away.
- Lack of flexibility. Only DNA binding sites can be produced on the array.
- Lack of variety. There are only a handful of different chips at present. All the tests have to be designed by the chip maker, making it impossible to add new tests to an existing system.

Luminex Corporation's FlowMetrix system makes use of coded microspheres, 6.5 μm in diameter. Each bead incorporates red and orange fluorophores to make up the code. Eight different intensities are possible, allowing 64 different bead types. A green fluorescent marker is used for the probes. The disadvantages of this system are:

- Relatively small number of codes – unsuitable for DNA probes
- Multi-wavelength optics required
- Systems are based around expensive ($100,000) multi-purpose flow cytometers

This invention combines the advantages of the Affymetrix GeneChip (~10,000 independent tests at once) and the Luminex FlowMetrix system (flexible, customisable test panels, real-time test results, low-cost consumables). However, the cost of this system is likely to be substantially lower, because of the relatively simple nature of the code readout system. This opens up the possibility of the widespread use of readers in hospitals and doctors' surgeries, for rapid screening purposes.

This invention describes a system for carrying out massively parallel multiple bioassay tests in a low-cost, fast and convenient manner. The scheme involves making a suspension containing many thousands of different types of micro-machined coded labels (micro-labels), each code carrying a different biochemical test. In one implementation, a novel reader system will draw through thousands of micro-labels per second, reading both the code and the result of the test. In this case, the code will be in the form of a transmission optical bar code, whilst the test result might typically be measured by the degree of fluorescence, or a colour change.

A specific embodiment of the invention will now be described by way of example with reference to the accompanying drawings in which:

Figure 1. Linear micro-label

Figure 2. How fluorescent markers become attached to micro-labels during bio-assay test

Figure 3. Silicon-on-Insulator-based label fabrication process

Figure 4. Schematic of linear label reading system

Figure 5. 2D labels

Description of embodiment

This embodiment has three main components

- Micro-miniature coded labels (micro-labels) shown in Figure 1
- Fluid handling system to allow rapid, serial reading of the micro-labels
- Optics and signal processing to read the micro-label code and the amount of fluorescence (Figure 4)

In this embodiment, the micro-labels consist of micro-machined miniature optical bar codes, made from silicon, using conventional VLSI techniques, with minimum feature sizes of around 2 μm. To enable accurate reading of the smallest label, they are made as thin strips with holes cut away to encode the data. Each micro-label is about 100 μm long by 10 μm wide by 1 micron thick, and is capable of storing up to 100,000 different codes This is shown in Figure 1. Around 10 million such micro-labels can be fabricated on a single 6-inch diameter substrate, using relatively conventional optical lithography. A number of low cost fabrication methods are possible, borrowing ideas from industries such as amorphous silicon solar cell and LCD display fabrication. Figure 3 shows a fabrication process based on Silicon-on-Insulator material.

Each code will uniquely identify a unique biochemical test, or probe. The probe is incorporated as part of the micro-label using well known techniques to coat the label with a particular DNA sequence, protein or antibody, which will bind to a specific target. The sample under test is labelled with a fluorescent marker, and incubated with a suspension of a mixture of the coded micro-labels. When the labelled target molecules bind to the antibody or DNA on a micro-label, the micro-label then becomes fluorescent, leading to a suspension of labels, some of which will have fluorescent groups attached (Figure 2).

A typical application might use a suspension of probes for a number of different antibodies (e.g. hepatitis, HIV). A reading system would take a drop of blood from a patient, label it with fluorescent marker, and incubate this with the probe suspension for a few minutes. The suspension would then be fed through a microfluidic detector system.

This will be a design that induces the micro-labels to flow down the centre of the tube in the central reading zone. There are two possible approaches to this – one is based on microfluidics alone, the other combines this with some magnetic material on the label and suitable coils and magnets to orient and position the label. In the second case, the label can be aligned with the flow direction by applying an axial magnetic field. This will enable the micron-scale bar code data to be read rapidly with very simple optics and signal processing. The pattern of holes on the label will be read by arranging for the label to intersect two orthogonal focused beams of light. The holes in the label will modulate the transmitted intensity as the label passes through, generating a serial stream of information that can be analysed in the same way as a conventional bar code. Simultaneously, the degree of fluorescence will be measured using a separate detector, and this will be correlated with the code on the label (Figure 4). High N.A. optics are needed to achieve the desired resolution. Even so, the optics and fluid handling required will be substantially simpler than in a conventional flow cytometer, where both scattering and multi-wavelength fluorescence analysis are normally used.

Once sufficient micro-labels have been read, the reader calculates the results of all the tests. For example, with blood samples, this would be a list of the disease strains found. In practice, each probe suspension would contain many copies of each of the desired tests to ensure a robust result. Washing and separating the blood from the micro-labels should be unnecessary because the micro-labels will be used to gate the fluorescence signals. Only very small amounts of sample and reagents are required, reducing the operating costs of the system.

The commercial products can be grouped into a number of categories:

- Suspensions of single-code micro-labels, prepared for probe attachment
- Suspensions of single-code micro-labels, with attached antibody, protein or DNA probe
- "Test-panels" of a large number of differently-coded micro-labels each with a unique probe
- Reading systems such as a desktop reader

Alternative features and embodiments

The embodiment above discusses a linear label with a code read by optical transmission. Alternative linear designs without holes in the label include:

- stripes of different (spectral) reflectivity or colour
- stripes of dielectric layers of one or more different thicknesses
- gratings with different pitches

An alternative is to use a 2D pattern, and fabricate labels which are closer to being square or rectangular, rather than linear. These labels require a different reading system, based on filtering or spinning out the label suspension onto a flat substrate. Labels incorporating magnetic material can use magnetic separation. This is useful if the sample being tested contains solid matter of similar size to the labels which could contaminate the results. The labels can then be read using a standard microscope system, either in transmission (for labels with holes) or with epi-illumination. This has the advantage of defining the focal plane accurately, at the expense of removing the transport mechanism for moving labels through the reader system.

Coding schemes similar to conventional 2D barcodes can be used. The outline of the label can be used to give orientation information. Some examples of different coding arrangements are given below.

Most codes are based around a grid of possible sites for holes on the label. The orientation is determined by a cut-out on the outside of the label. If the minimum feature size and the placement accuracy are both f, the grid has a pitch of f, and a selection rule is used which prevents holes from being placed closer than $2f$. For example, using a 6x6 grid with a pitch of 2.5 μm, the label with cut-out is 22.5x20 μm, and the number of codes is in excess of 10^5 (Figure 5a).

If the manufacturing process can tolerate *placement* of the holes to a higher precision than the minimum feature size (e.g. placement to 0.83 μm, with a minimum feature size of 2.5 μm), then higher data densities can be achieved: for example, 10^5 codes can be produced on a grid of 12x12 sites at a pitch of 0.83 μm, with a selection rule preventing sites which are closer than 5 μm from being occupied. This gives a label with cut-out which is 19x16.5 μm (Figure 5b).

Figure 5c shows a very simple, intuitive 2D coding scheme. There are 5 rows, each with ten possible positions for a single hole in each row, at a pitch of 1 μm. This gives a total of 10^5 codes. The orientation of the label is determined by a cut-out on the edge.

CLAIMS

1. Small labels held in suspension incorporating a spatially varying pattern for identification.

2. Optical reader for reading codes on labels in 1

3. Labels where pattern is a barcode

4. Labels where pattern is linear barcode

5. Labels in 1 using transmission optics

6. Labels in 1 incorporating biochemical test

7. As in 6, result indicated by fluorescence

8. Labels in 6 with reader (2) plus method for interrogating result of test

9. Method of making labels 1 by lithographic process

10. Method of bio assay

11. Fluid dynamic method of aligning labels with fluid flow

12. Magnetic method of aligning particles with fluid flow

13. Label as in 1 where pattern is a diffraction grating

~~14. Method of filtering out labels and reading codes on surface of filter~~

15. Method of magnetically separating particles

ABSTRACT

This invention relates to a micro-machined label incorporating a spatially varying pattern, capable of storing many thousands of codes, primarily for use in massively parallel bio-assay applications. The invention describes methods for making the labels, reading them, and using them for bio-assay purposes.

Sentec Ltd check box:

Pages of text	: 5
Pages of claims	: 1
Pages of abstract	: 1 (this one)
Pages of figures	: 5

Initial Application

INVENTION TITLE:

Micro-fabricated coded labels, reading systems and their applications

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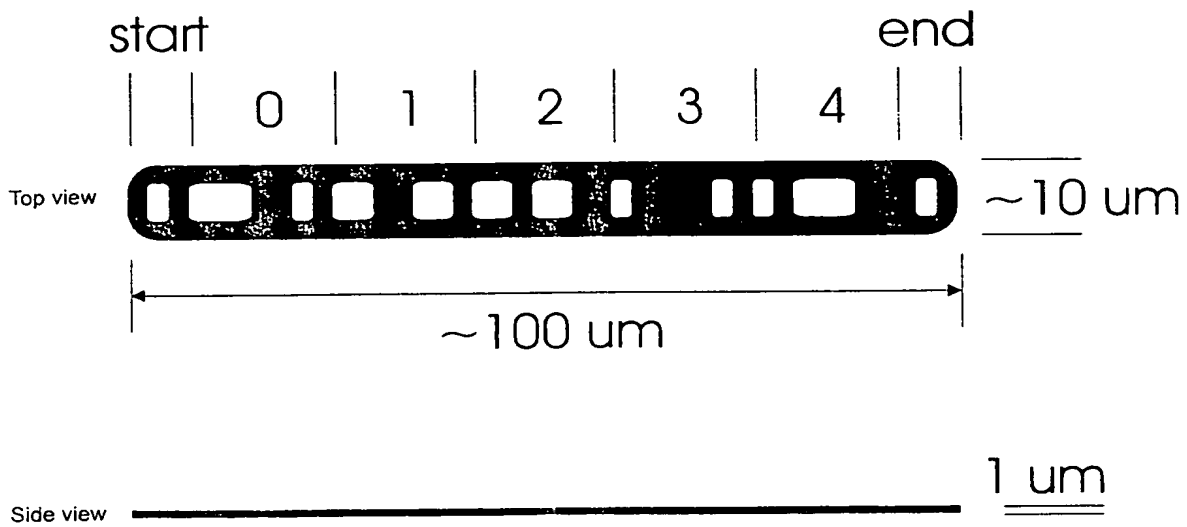
FIGURES



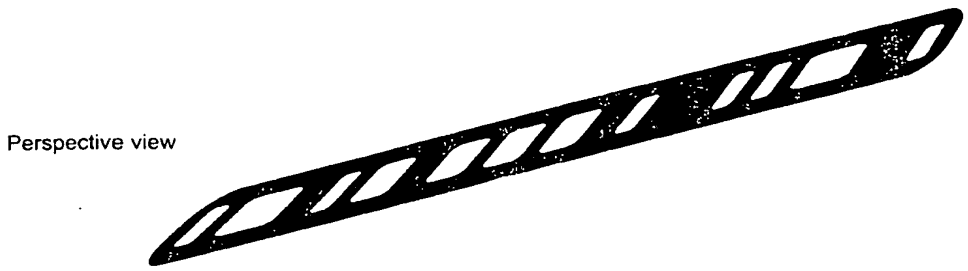


Figure 1.

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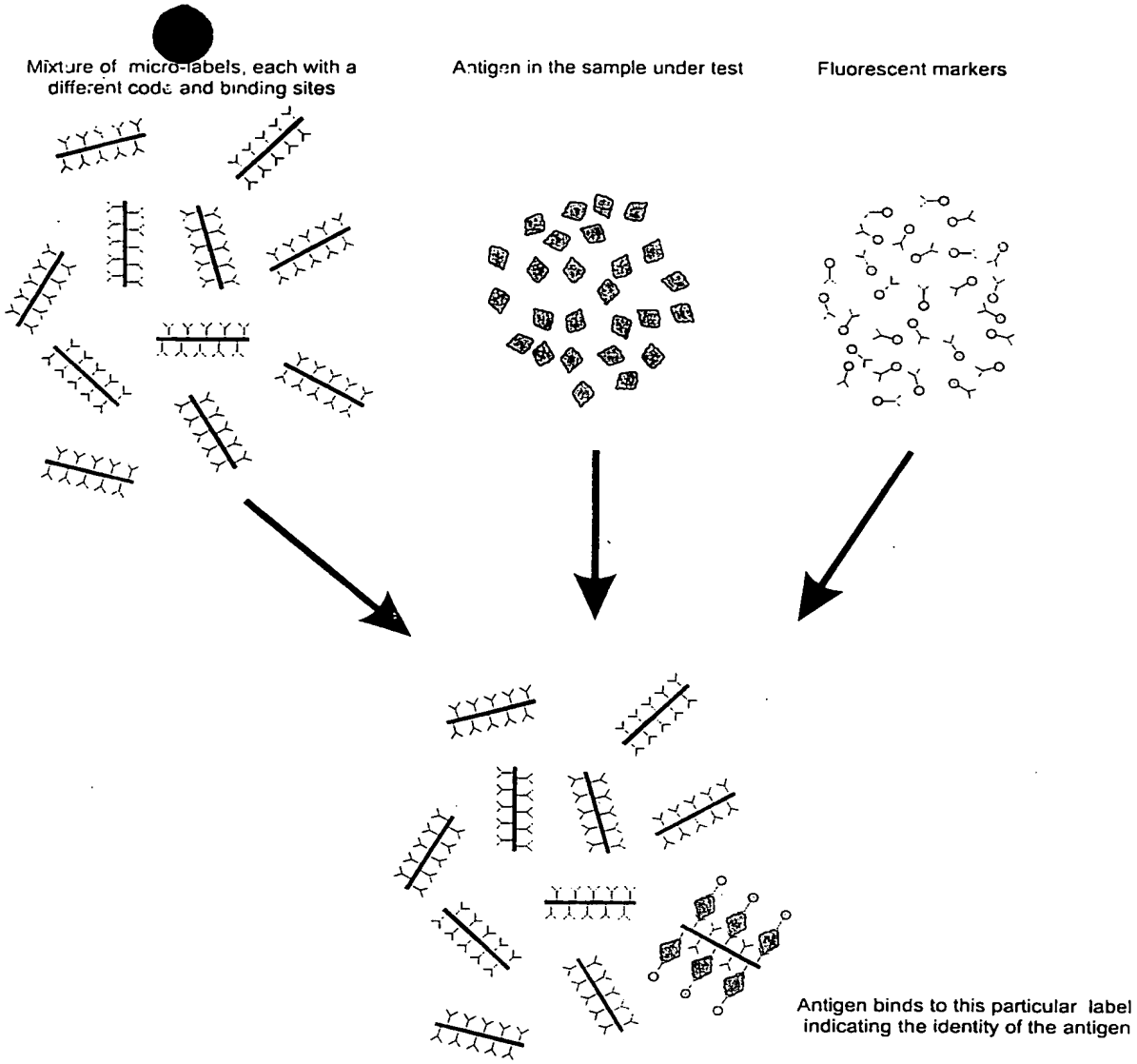
Mixture of micro-labels, each with a different code and binding sites
Antigen in the sample under test
Fluorescent markers
Antigen binds to this particular label indicating the identity of the antigen

Figure 2.

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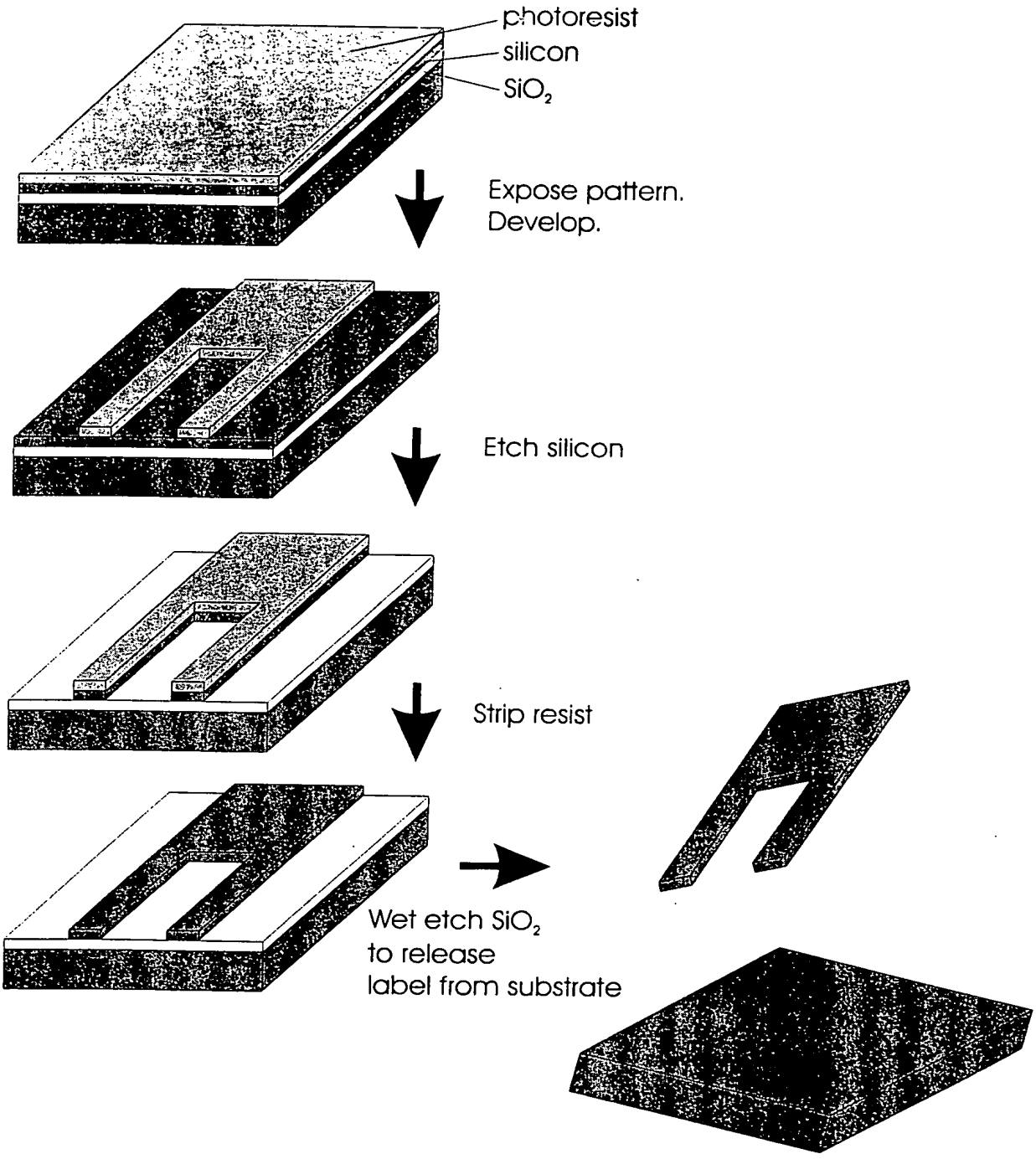
photoresist
silicon
SiO_2
Expose pattern.
Develop.
Etch silicon
Strip resist
Wet etch SiO_2
to release
label from substrate

Figure 3.

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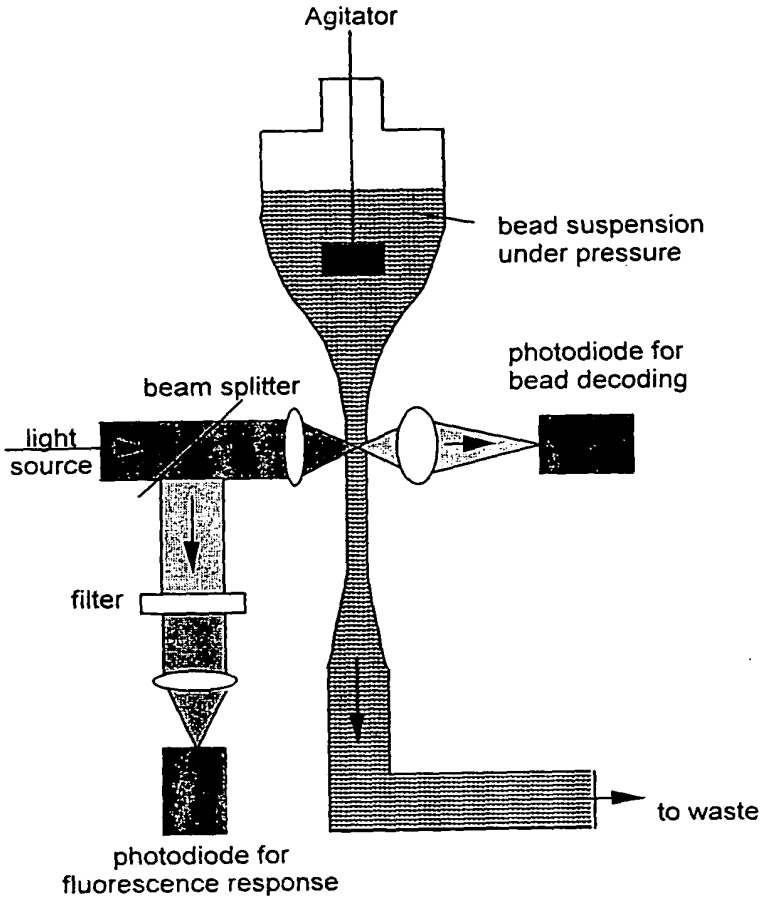
Agitator
bead suspension
under pressure
photodiode for
bead decoding
beam splitter
light
source
filter
to waste
photodiode for
fluorescence response

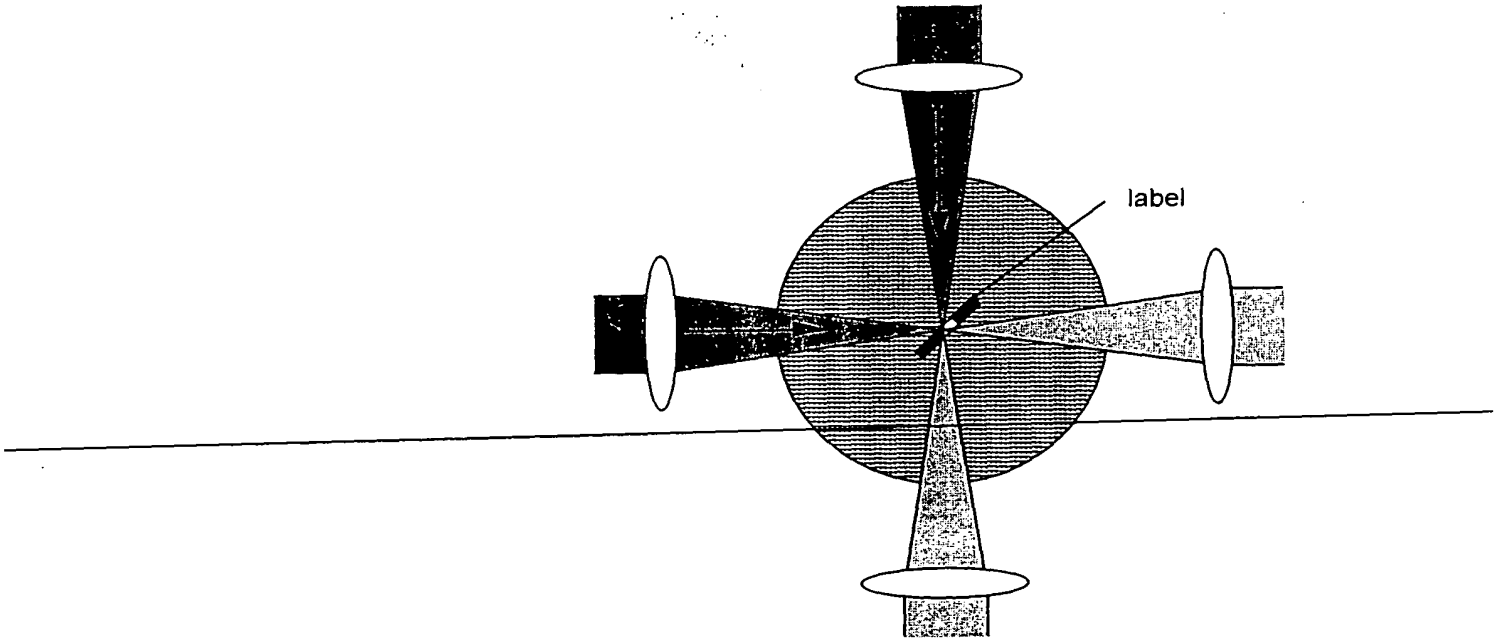
label

Figure 4.

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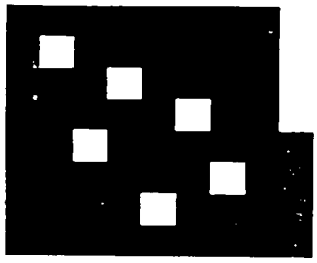
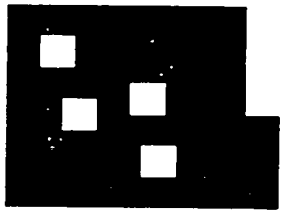
a. 2.5 um grid



b. 0.83 um grid



c. 5 rows
1um increment

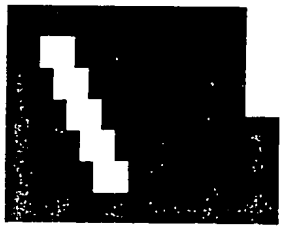


Figure 5.

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